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Modulation of aggression in male mice: influence of group size and cage size

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Abstract

Aggression in group-housed male mice is known to be influenced by both cage size and group size. However, the interdependency of these two parameters has not been studied yet. In this study, the level of aggression in groups of three, five, or eight male BALB/c mice housed in cages with a floor size of either 80 or 125 cm^2/animal was estimated weekly after cage cleaning for a period of 14 weeks. Furthermore, urine corticosterone levels, food and water intake, body weight, and number of wounds were measured weekly. At the end of the experiment, tyrosine hydroxylase (TH) activity, testosterone levels, and weight of spleen, thymus, testes, and seminal vesicles were determined. Results indicate a moderate increase of intermale aggression in larger cages when compared to the smaller cages. Aggression in groups of eight animals was considerably higher than in groups of three animals. The increase of agonistic behavior was observed both in dominant and subordinate animals. Physiological parameters indicate differences in stress levels between dominant and subordinate animals. It is concluded that aggressive behavior in group-housed male BALB/c mice is best prevented by housing the animals in small groups of three to five animals, while decreasing floor size per animal may be used as a temporary solution to decrease high levels of aggression in an existing social group.

Keywords: Aggression; Mice; Cage size; Crowding; Group size; Welfare

1. Introduction

When male laboratory mice are housed in groups, they will show aggressive behavior towards each other in order to develop and maintain a social hierarchy. The level of aggression depends on strain and age of the mice and environmental factors such as cleaning procedures [1], group size, and cage size. Earlier studies mostly agree that aggression increases with increasing group size and decreasing floor area per animal [2–6], although some studies report no effect of cage size on aggression [7,8] or even a reverse effect [9]. The two factors (group size and cage size), however, have usually not been investigated independently, and in most studies, cage sizes were overall 5–50 times the size of a standard laboratory cage. Furthermore, aggressive behavior was studied for only a brief period of time (1–8 days) when the male mice had already reached adulthood. Studies in which the development of aggression in weanling groups is monitored are rare [1].

Husbandry procedures such as cage and group size have a major impact on the well-being of laboratory animals, and the research they are used for as psychologically and physiologically healthy animals is a precondition for reliable experimental results [10]. New insights in social behavior of laboratory animals have made clear that a revision of existing guidelines on cage and group size is desirable [11].

Both in the Resolution on Accommodation and Care of Laboratory Animals [11,12] and in the FELASA Report of the Rodent Refinement Working Party [13] cage size is mentioned as one of the main areas that needs to be studied in more detail. At present, recommended cage sizes for mice are solely based on weight of the animals (10–40 g) and the number of animals per cage (1–30). Age, gender, or social behavior of the mice is not taken

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into account. Recommendations on population density or group composition are not included.

To investigate the differential effect of group size and cage floor size on the level of aggression in male laboratory mice, an experiment was designed in which both behavioral and physiological effects were studied in male mice housed in groups of three different sizes and at two different population densities. Results may aid in formulating recommendations that can be included in revised guidelines with regard to cage and group size of male mice.

2. Methods

2.1. Animals and husbandry

Ninety-six male mice (*Mus musculus*) of the BALB/cAnNCrlBr strain were used. Mice of the BALB/c strain may show substantial intermale aggression, although severe aggression leading to death if mice are not separated is rare. The BALB/c strain is therefore a good model for the study of intermale aggression in existing social groups. The mice were randomly divided in groups of three ($n=6$), five ($n=6$), or eight mice ($n=6$) and housed in wire-topped clear Perspex cages provided with 15 g/100 cm^2 sawdust (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Cages were cleaned weekly. Tap water and food pellets (RMH-B, Hope Farms, Woerden, The Netherlands) were provided ad libitum. The animal room had a controlled photoperiod (lights on between 07:00 and 19:00 h), temperature (23–24°C), relative humidity ($60\pm5\%$), and ventilation (18–20 air changes/h).

At the start of the experiment, the mice were 7 weeks old. The animals were individually marked on the fur with a black waterproof marker. The mark was renewed weekly.

2.2. Procedure and behavioral data collection

At the age of 7 weeks, mice were housed either at 80 (three groups of each group size) or 125 cm^2/mouse (three groups of each group size). During 14 weeks thereafter, cage size was alternated weekly after cage cleaning. Cages used were Makrolon type II (375 cm^2), type III (825 cm^2), and type 2154 F (945 cm^2; Tecniplast, Milan, Italy). To adjust cage size, all cages were provided with a flexible Perspex wall. In addition, Makrolon type 2154 cages were provided with a Perspex floor insert to adjust cage height. Prior to cage cleaning, food and water were weighed and refreshed; animals were weighed; and wounds on tail, back, and genitals were counted. Immediately after transferring the mice to their new environment, their behavior was recorded on videotape for a period of 30 min. Due to restrictions in the experimental setup, the number of cages cleaned and videotaped simultaneously was limited to four. To minimize influence of time of day on behavior, order of cages cleaned and recorded was altered weekly according to a previously established randomization procedure.

2.3. Behavioral analysis

Latency until first agonistic encounter, frequency and duration of agonistic encounters were scored from videotape. Behaviors interpreted as agonistic were several offensive behaviors such as vigorous sniffing of head, tail, or genitals of the opponent, tail rattling, chasing, biting and fighting (wrestling while biting, mainly in the flanks), and several defensive behaviors such as adopting upright and sideways defensive posture, flee and active defense. Encounters that included biting were marked separately (escalations), as well as encounters that included fighting (fights). The identities of the males involved in an encounter were noted. A male was said to initiate an agonistic encounter when it showed the first agonistic behavior. A male was said to win an encounter when its opponent showed submissive behavior terminating the agonistic encounter. Dominant status was allocated to one animal in each group that initiated and won the highest number of encounters. Subordinate status was allocated to two animals in each group that were attacked most (sub+) or least (sub −).

2.4. Urine collection and corticosterone analysis

Every week, urine samples were collected for corticosterone and creatinine analysis. Between 09:00 and 10:00 h, mice were placed individually in plastic buckets (1.1 l; Emergo, Landsmeer, The Netherlands) provided with a plastic salad dish (250 cc, depa) and a wire top until the mice urinated, but no longer than 50 min. Urine was then collected with a syringe and stored in polypropylene tubes at −20°C (method described by Dahlborn et al. [14]). Urine of six groups was collected simultaneously at 2, 3, or 4 days after cage cleaning, according to a randomized block procedure. Corticosterone levels were measured using a solid-phase ^{125}I radioimmunoassay (CAC Rat Corticosterone TKRC1, Diagnostic Products, LA). Creatinine concentrations were determined with the use of a commercial test combination (Creatinine, MA-KIT 10 ROCHE, Roche Diagnostics) on a COBAS-BIO autoanalyzer (Hoffmann-La Roche, Mijdrecht, The Netherlands).

2.5. Organ weights, testosterone levels, and tyrosine hydroxylase (TH) activity

At the age of 20 weeks, three animals of each group (dominant, sub+, and sub −) were euthanized simultaneously by three animal technicians between 09:00 and 12:00 h by decapitation to enable blood collection without contamination of anaesthetic compounds. Trunk blood was collected in ice-cooled 1.5 ml reaction vessels containing 50 iu heparin/ml blood. Blood was centrifuged (3000 rpm, 25

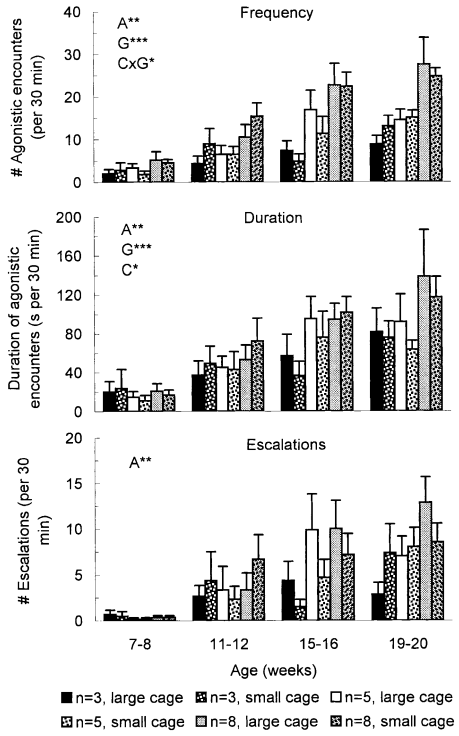

Fig. 1. Frequency of agonistic encounters (top), duration of agonistic encounters (middle), and number of escalated encounters (bottom) during 30 min after cage cleaning of male mice, specified for four age categories, large or small cages (125 and 80 cm^2, respectively), and group sizes of three, five, or eight mice. A: age effect; C: cage size effect; G: group size effect. $*P<.05$; $**P<.01$; $***P<.001$.

min at 20°C) and serum stored at −20°C until assayed. Testes, spleen, thymus, and seminal vesicles were dissected and weighed (testes and seminal vesicles in pairs). Adrenals were dissected, individually shock-frozen in 5 mM Tris–HCl buffer (pH 7.2), and stored at −70°C. Serum testosterone concentration was measured using a solid phase ^{125}I radioimmunoassay (CAC Total Testosterone TKTT, Diagnostic Products). TH was measured in adrenals using a tyrosine-^{14}C-assay (method described by Witte and Matthaei [15]).

2.6. Statistical analysis

Behavioral data, as well as body weight, food and water intake, organ weights, TH activity, and serum testosterone levels, were analyzed using a multivariate analysis of variance for repeated measures with multiple comparisons. Where necessary, data were logarithmically transformed to better fit the normal distribution or to improve homogeneity of variances. For nonparametric behavioral data (fights), the Friedman test was applied. Furthermore, Pearson's correlation (r) was calculated between the total amount of aggression in a group (corrected for group size by dividing by $n-1$) and several physiological parameters (organ weight, TH activity, and testosterone level). Number of wounds was analyzed using analysis of variance with negative binomial error. Urine corticosterone data were logarithmically transformed and analyzed using a mixed-effects analysis of variance, with mouse identity as random effect. For all tests, Bonferroni correction was applied where necessary. When status of the animal was taken into account, only data of three animals in each group (dominant, sub+, and sub−) were used for analyses. Number of wounds and corticosterone analyses were carried out with aid of S-plus 2000 Professional Release 2 (1988–1999, MathSoft). All other statistical tests were carried out with aid of SPSS for MS Windows Release 9.0 (Chicago, IL).

3. Results

3.1. Behavior

All behavioral scores revealed significant age effects. Frequency (fr) and duration (du) of aggression, escalations (es) and duration of escalations (de) all increased with increasing age of the mice [$F_{fr}(6,10)=13.787$; $F_{du}(6,10)=7.231$; $F_{es}(5,11)=6.682$; $F_{de}(5,11)=9.865$; $P_{all}<.01$], while latency to first agonistic encounter decreased with age [$F(6,10)=7.996$, $P<.01$]. Frequency, duration, and escalations are presented in Fig. 1. Furthermore, group size and cage size effects were present in frequency and duration of agonistic encounters (Fig. 1). Both increased with increasing group size [$F_{fr}(2,15)=24.674$, $P<.001$; $F_{du}(2,15)=3.880$, $P<.05$]. Multiple comparisons reveal that differences in frequency were mainly due to differences between groups of eight mice and groups of three or five mice (Bonferroni $P<.001$). Differences in duration are most obvious between groups of three and eight mice (Bonferroni $P=.055$). Furthermore, duration of agonistic encounters was significantly higher in larger cages [$F(1,15)=4.669$, $P<.05$], and frequencies revealed a significant cage size × group size interaction [$F(2,15)=6.106$, $P<.05$]. In groups of five mice, frequency of agonistic encounters was higher in larger cages, while in groups of three and eight mice, there were no differences between large and small cages. Fights between mice were

Table 1
Total amount of fights observed in 14 weeks during 30 min after weekly cage cleaning, specified for groups of three, five, and eight mice and for large and small cages

Observed fights		
	Cage size	
Group size	Large[a]	Small[b]
3	32	37
5	49	48
8	60	76

[a] Floor size 125 cm^2/animal.
[b] Floor size 80 cm^2/animal.

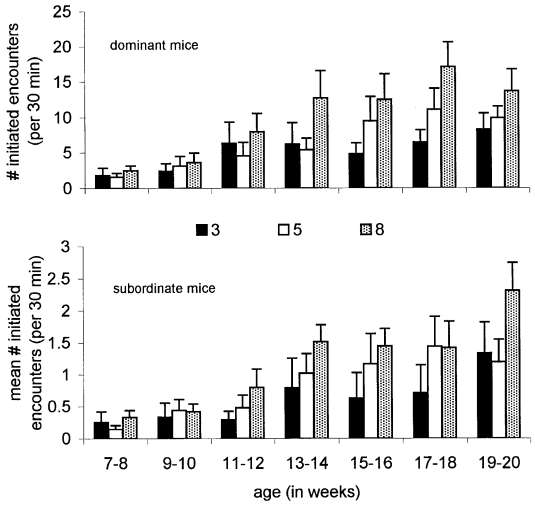

Fig. 2. Number of aggressive encounters per 30 min initiated by dominant mice (mean ± S.E.M.; top) and subordinate mice (mean ± S.E.M.; bottom) for seven age categories and three group sizes.

quite rare and increased slightly with age. No differences in fights between groups or cages of different sizes could be revealed statistically (Table 1).

To investigate the differences in agonistic behavior between groups of three, five, and eight mice more closely, behavioral data were scrutinized in some detail. The following comparisons were made.

1. The highest number of agonistic encounters that one animal (sub+) in each group was subjected to in any given week.
2. The highest number of agonistic encounters that one animal (dominant) in each group initiated in any given week.
3. The mean number of encounters initiated by the subordinate animals in any given week, i.e. total of two subordinates divided by 2 (groups of three), total of four subordinates divided by 4 (groups of five), or total of seven subordinates divided by 7 (groups of eight).

For the first comparison, no significant group size effects were found. For Comparisons 2 and 3, a significant group size effect was apparent [Fig. 2; $F_2(2,15) = 3.788$; $F_3(2,15) = 3.814$; $P_{2,3} < .05$]. Contrast results show that agonistic encounters between nondominant animals occurred more often in groups of eight than in groups of three mice (Bonferroni $P < .05$). Furthermore, the dominant animal showed more agonistic behavior in groups of eight than in groups of three mice (Bonferroni $P = .055$). For both comparisons, the frequencies in groups of five mice were intermediate and did not differ significantly from either groups of three or groups of eight mice.

3.2. Wounds

The majority of wounds (94%) were found on the base of the tale and the back of the mice. Incidentally, wounds were found on genitals, paws, or ears. To include the status of the mice in the test, only wounds of the dominant, the most attacked subordinate, and the least attacked subordinate were taken into account. Other wound data were excluded from analysis. Statistical analysis revealed a clear effect of age, group size, cage size, and status (Fig. 3). The number of wounds increased between the age of 7 and 10 weeks; after which, it stabilized ($P < .001$). In groups of five and eight mice, the mice are significantly more wounded than in groups of three mice (Bonferroni $P_{3-8} < .05$; Bonferroni

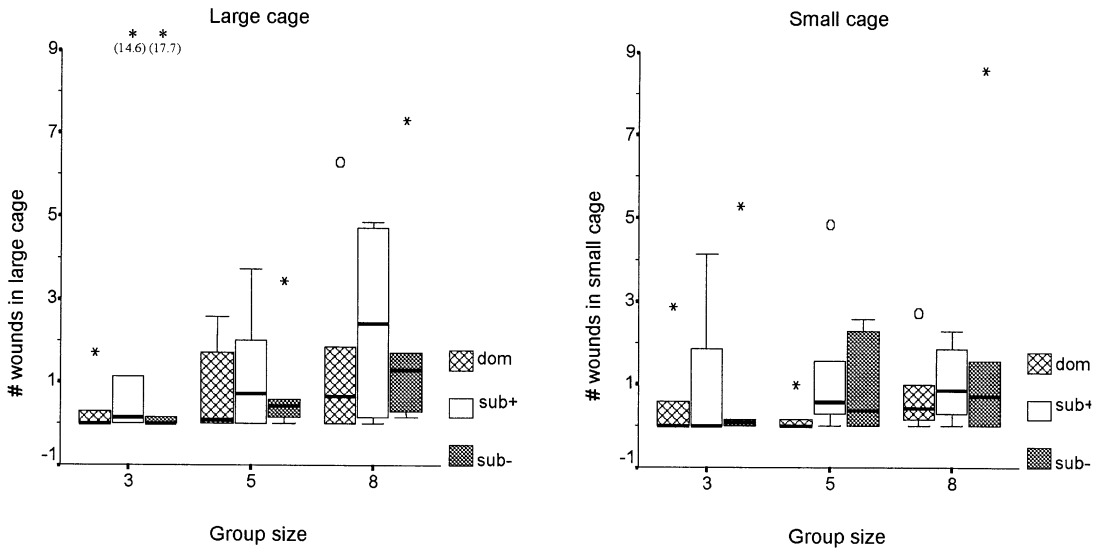

Fig. 3. Number of wounds of dominant, sub+, and sub − mice of three different group sizes, separated for large cages (left) and small cages (right). Box plots show median values with interquartile ranges, highest and lowest nonoutlying values. °Indicate outliers; * indicate extreme outliers (number of wounds is mentioned if the extreme falls outside the figure range).

Table 2
Food and water consumption per mouse per week specified for two age categories, large or small cages, and group sizes of three, five, or eight mice

		Food intake (g; mean ± S.E.M.)		Water intake (ml; mean ± S.E.M.)	
		Cage size		Cage size	
Age (weeks)	Group size	Large[a]	Small[b]	Large	Small
8–13	3	21.1 ± 0.4	21.0 ± 0.4	23.0 ± 1.1	22.7 ± 0.7
	5	21.8 ± 0.2	21.0 ± 0.3	22.2 ± 0.3	21.4 ± 0.4
	8	22.3 ± 0.3	21.7 ± 0.3	22.7 ± 0.6	22.3 ± 0.5
14–19	3	21.9 ± 0.3	21.8 ± 0.4	23.5 ± 1.0	22.1 ± 0.7
	5	23.6 ± 0.4	22.7 ± 0.3	23.3 ± 0.8	21.9 ± 0.6
	8	25.3 ± 0.4	24.2 ± 0.5	24.1 ± 0.7	23.5 ± 0.6
Significance		A***; C***; G(*); A × G**		A***; C**	

A: age effect; C: cage size effect; G: group size effect.
[a] Floor size 125 cm^2/animal.
[b] Floor size 80 cm^2/animal.
(*) $P < .1$.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

$P_{3-5} < .001$). Furthermore, the number of wounds counted was higher in the larger cages ($P < .01$), and most attacked subordinates had significantly more wounds than dominant mice (Bonferroni $P < .01$) while least attacked subordinates were intermediate.

3.3. Body weight and food and water intake

Body weight of the mice ranged from 13.58 ± 0.11 g at the start to 26.23 ± 0.17 g at the end of the experiment. No differences were found in body weight between animals of

Table 3
Weight of several organs corrected for body weight, TH activity, and serum testosterone levels specified for group sizes of three, five, or eight mice and status of dominant, most attacked subordinate, or least attacked subordinate and their correlation with aggression

Organ weights, TH activity, and testosterone levels (mean ± S.E.M.)					
	Group	Status			Correlation with
Parameter	size	Dominant	Sub +	Sub −	aggression
thymus (mg/g body weight)	3	1.35 ± 0.14	1.32 ± 0.09	1.40 ± 0.15	n.s.
	5	1.14 ± 0.10	1.36 ± 0.08	1.59 ± 0.14	
	8	1.22 ± 0.11	1.20 ± 0.08	1.34 ± 0.11	
seminal vesicles[a)] (mg/g body weight)	3	7.95 ± 0.69	7.97 ± 0.51	7.57 ± 0.38	n.s.
	5	7.80 ± 0.54	6.86 ± 0.35	6.64 ± 0.68	
	8	7.23 ± 0.55	7.48 ± 0.62	7.38 ± 0.67	
spleen (mg/g body weight)	3	4.66 ± 0.49†	5.60 ± 0.81	5.00 ± 0.67‡	r_{sub+}=0.630**
	5	4.28 ± 0.59†	4.42 ± 0.49	5.38 ± 0.33‡	
	8	4.18 ± 0.36†	5.12 ± 0.49	4.95 ± 0.52‡	
testes[a)] (mg/g body weight)	3	6.85 ± 0.30	7.53 ± 0.21	7.22 ± 0.23	$r_{dom} = -0.649$**
	5	6.98 ± 0.27	6.91 ± 0.20	7.28 ± 0.07	
	8	7.14 ± 0.13	6.95 ± 0.28	6.70 ± 0.43	
TH activity (nmol × h^{-1} × adrenal pair)	3	5.25 ± 0.47§	5.99 ± 0.46§	4.30 ± 0.26#	r_{sub+}=0.419(*)
	5	7.74 ± 1.81§	4.81 ± 0.68§	3.74 ± 0.40#	
	8	6.26 ± 0.66§	6.01 ± 0.60§	5.31 ± 1.00#	
testosterone (ng/ml) [median]	3	13.22 ± 4.97 [11.41]	7.72 ± 5.17 [0.55]	15.73 ± 6.84 [13.65]	$r_{sub+} = -0.451$(*)
	5	2.46 ± 0.91 [1.77]	5.07 ± 3.81 [0.55]	6.85 ± 5.24 [1.78]	$r_{sub-} = -0.472$*
	8	12.42 ± 6.16 [5.57]	4.48 ± 3.59 [0.66]	6.68 ± 4.35 [1.80]	

[a)] Weighed in pairs.
†-‡ (*): $P < .1$.
§-#, *: $P < .05$.
**: $P < .01$.

different group or cage sizes or between dominant and subordinate (sub+ or sub −) animals within groups. Food and water data are summarized in Table 2. The mice ate and drank significantly more with age [$F_{food}(5,11) = 40.787$, $P < .001$; $F_{water}(5,11) = 3.809$, $P < .05$]. Furthermore, the mice ate and drank more when housed in the larger cages [$F_{food}(1,15) = 14.093$, $P < .01$; $F_{water}(1,15) = 9.382$, $P < .01$]. Group size affected eating overall slightly [$F(2,15) = 3.247$, $P < .1$], and there was an obvious group size × age interaction effect: in groups of eight mice, the increase of food intake with time was more pronounced than in groups of three mice, while groups of five mice were intermediate [$F(10,24) = 3.324$, $P < .01$]. Water intake was not affected by group size.

3.4. Organ weights, hormone levels, and TH activity

All organ weights were corrected for final body weight; weights of seminal vesicles and thymuses were corrected for animal technician, as an unwanted effect of animal technician (probably due to differences in removal of fat tissue) was present. Results of organ weights, TH activity, and testosterone levels and their correlation with aggression are summarized in Table 3. Group size did not affect any of the physiological parameters measured, while hierarchy affected several parameters. Animals that were least attacked had slightly heavier spleens than dominant animals, although not significant ($P < .1$). Status had a significant effect on TH activity [$F(2,16) = 4.655$, $P < .05$]. TH activity was lower in animals that were least attacked compared to TH activity in dominant animals (Bonferroni $P < .05$) and animals that were attacked most (Bonferroni $P < .05$). Urine corticosterone levels corrected for creatinine levels (Co/Cr ratio) showed a significant quadratic time effect. Initially, the mean ± S.E.M. Co/Cr ratio of $9.49 \times 10^{-6} \pm 0.60$ at age 8–9 weeks decreased to $8.80 \times 10^{-6} \pm 0.89$ at age 12–13 weeks where after it started to rise again to 11.59 ± 0.57 when the animals were 18–19 weeks old ($P < .001$). Level of aggression was found to correlate with TH activity of most attacked mice ($r = .419$; $P < .1$), testosterone levels of most and least attacked animals ($r_{sub+} = -.451$; $P < .1$; $r_{sub-} = -.472$; $P < .05$), spleen weight of attacked mice ($r = .630$; $P < .01$), and testes weight of dominant mice ($r = -.649$; $P < .01$). Any other correlation with aggression was not significant (Table 3).

4. Discussion

4.1. Age effects

Both behavioral and physiological variables tested during the course of the experiment showed significant age effects. Not surprisingly, aggression increased when the mice grew older; the mice ate and drank increasingly more with age and weighed increasingly more. For the variables mentioned above, the main age effect was linear. For body weight, there was an additional quadratic effect, i.e. the increase in body weight decreased with age. The best model to fit the age effect for corticosterone levels was a quadratic effect. Initially, corticosterone levels decreased, reached a bottom when the mice were around 11–12 weeks of age, and thereafter started to rise again. An obvious explanation would be that after grouping, corticosterone levels were increased due to social stress of encountering unfamiliar cage mates. Thereafter, corticosterone levels began to drop, as the hierarchy within the groups became stabilized. Indeed, Bronson [16] reported an increase in corticosterone after grouping, followed by a decline, as groups became stable. The secondary increase in corticosterone paralleled the observed increase of aggression that we observed. Goldsmith et al. [17] also reported an increase in corticosterone due to an increase in fighting.

4.2. Cage size effects

The effects of cage size on agonistic behavior, occurrence of wounds, and physiological data were found to be small but consistent. The duration of agonistic encounters was significantly higher in larger cages (Fig. 1). Furthermore, the number of agonistic encounters in groups of five was significantly higher in larger cages compared to smaller cages. Accordingly, the number of wounds was higher in larger cages compared to smaller cages (Fig. 3).

In general, the decreased levels of aggression and number of wounds in smaller cages might be explained by a "crowding" effect. Several studies have reported that crowding causes a decrease in aggression [9,18–20]. However, in other studies, no effect of population density on aggression [7] or indeed an *increase* of aggression as a result of decrease in space allowance has been reported [3,5,21]. This apparent discrepancy may be explained by a curvilinear relationship between crowding and agonistic behavior [19,22]. It can be argued that in first instance aggression will increase with increasing population density, as invasions of the dominant's territory occur more often. When density is extremely high, however, the available space may be too small for the dominant mouse to form a defendable territory, leading to a decrease in aggression [23]. Indeed, in the studies reporting an increase of aggression with decreasing space [3,5], mice were not housed in standard laboratory cages but were observed in areas between 360 cm^2 and 1.5 m^2/mouse (3–200 times the sizes used in this study).

Although there was no difference in weight gain, the mice ate and drank more when housed in larger cages. This is in accordance with Chvédoff et al. [24] who found a decrease in both food and water consumption in groups of higher density while body weight did not differ. Peters and Festing [25] did not find any effect of crowding on body weight or adrenal weight in mice and concluded that

mice are relatively insensitive to crowding. However, floor size was ranged from 27 to 55 cm^2/mouse, and crowding was induced by increasing the number of animals rather than decreasing floor size. It may be argued that in the study by Peters and Festing [25], all mice were observed while being accommodated in a crowded situation. Urine corticosterone levels were not influenced by cage size. In several studies, higher corticosterone levels are reported as a result of crowding. In each of these studies, however, group size increased while space allowance decreased [6,26,27].

4.3. Group size effects

In this study, the effects of group size on aggression were the most pronounced. Aggression increased substantially with increasing group size. These results are in accordance with Butler [4] who found that doubling of the population number of laboratory-reared wild house mice while holding stocking density constant lead to an increase of aggression in mice. Close scrutiny of the data in the present study revealed that a higher level of aggression of both the dominant mouse and the subordinate mice in groups of eight mice caused the higher level of aggression. Furthermore, the number of wounds was higher in groups of five and eight mice than in groups of three mice (Fig. 3). These results suggest that larger groups are more restless than smaller groups. The dominant male showed more agonistic behavior probably to sustain its dominant status, while subordinate animals showed more agonistic behavior possibly to gain a higher status within the hierarchy. Indeed, Poole and Morgan [2] found that large groups of mice had a more unstable hierarchy than smaller ones and dominance status changed between animals more often. Cunningham [20], who studied hens in a similar setup to the present study, also found that in larger flocks, both the dominant and the subordinate hens showed more aggression than in smaller flocks.

No effects of body or organ weights, urine corticosterone levels, testosterone levels, or TH activity were found with respect to group size. A significant positive correlation in mice between corticosterone levels (in adrenals and serum, respectively) and group size was found by Gärtner and Benath [26] and Barnard et al. [6]. In both studies, however, floor size per mouse fluctuated for different group sizes, thus possible cage size × group size interaction effects can not be ruled out.

Animals in larger groups tended to eat more than those in smaller groups, especially when the mice grew older. Animals of a social species do not only eat to become satiated but also to fulfill the need of social contact. This phenomenon is known as social facilitation [28]. As the chances of one animal being triggered to eat by another animal that is eating in a group of eight mice is higher than in a group of three or five mice, mice in groups of eight mice may eat more often and for longer bouts.

4.4. Social status effects

For several physiological parameters, effect of social status (dominant, attacked subordinate, and less attacked subordinate) was measured. In concordance with the number of attacks scored, mice that were most attacked had most wounds, while dominant mice had least wounds and least attacked mice were intermediate. No age × status interaction effects were found, indicating that in general, hierarchy was stable in time. Indeed, only in one group of eight mice, dominant status was observed to shift between two mice. In none of the other groups, such clear shift was noted.

Body weight was not affected by social status. This seems to be in contrast to previous results [1], where we found that dominant animals were initially the lighter animals, while heaviest toward the end of the experiment. However, the mice in the latter study were several weeks younger at the start of the experiment, and by the time the dominant mice reached the age of 7 weeks (initial age of the mice in this experiment), they had already caught up on weight with their cage mates. Indeed, Jeppesen and Hansen [29] found a significant correlation between weight gain and rank in subadult mice (3 weeks) but not adult mice (13–16 weeks).

TH is an enzyme that mediates the transition from tyrosine to dopamine, a precursor for noradrenaline. It provides an estimate of relatively long-term activity of the adrenal gland [30]. We found that TH activity was high in dominant and most attacked subordinate animals and low in least attacked subordinate animals. This is in accordance with Haemisch and Gärtner [31] who found that dominant and active subdominant animals had higher TH activities, while TH activity of passive subdominant animals was lowest. Indeed, both maintaining dominance (α-males) or being defeated (ω-males) is stressful, while accepting a subordinate status without ever challenging the α-male may be less stressful (β-males; Ref. [32]). TH activity of attacked mice, but not dominant mice, seemed to correlate positively with aggression. For subordinate animals that are attacked often, the amount of aggression may indeed influence the level of stress they experience. Dominant animals, on the other hand, have more control over the situation, regardless of whether they are more or less overtly aggressive. Levels of stress of dominant mice may thus fluctuate less with levels of aggression. Indeed, Maengwyn-Davies et al. [33] found an increase in TH activity in mice that were exposed to aggressive mice.

Testosterone values of dominant mice were highest and those of attacked animals were lowest, although differences were not significant. Testosterone is known to be emitted in a pulsatile pattern [34]. This may account for the large variation in measurements within the group of mice studied here, thus obscuring any possible differences between dominant and subordinate mice. It is noteworthy, however, that the results are in accordance with previous findings [1] and with Bishop and Chevins [35] and

Barnard et al. [6] who also found higher though not significant testosterone values in dominant mice. Furthermore, we found a negative correlation between testosterone values of both attacked and nonattacked subordinate mice and aggression. Indeed, testosterone levels in rats have been shown to be suppressed by defeat and subordination [36,37]. Parmigiani et al. [38] showed that suppression of testosterone also occurs in subordinate mice that are not subjected to attacks. Spleens of least attacked subordinates were slightly heavier than those of dominant animals, although not significant, and spleen weight of attacked subordinates was positively correlated with aggression. Blanchard et al. [36,37] reported that continuous social stress increased spleen weight. The fact that testes of dominant mice were negatively correlated with aggression may be somewhat surprising. It may however be hypothesized that the dominant mice with most 'natural authority' (macho) have heavier testes and need to be less overtly aggressive to maintain dominance than those animals (with lighter testes) that repeatedly have to reinforce their dominant status.

5. Conclusions

When housing male mice as laboratory animals, the level of aggression can be influenced substantially by group size and, to a lesser extent, by cage size. A high level of aggression can be decreased by decreasing cage size. However, decreasing cage size as low as 80 cm^2/mouse may cause stress due to crowding. In this study, two cage sizes were compared. More cage sizes need to be tested to form a theory about the optimum cage size with respect to aggression and stress.

To prevent unacceptable levels of aggression or at least to slow down its development, it is advisable to keep male laboratory mice in small social groups (three to five animals per cage). The animals have less chance to be wounded severely in such small groups, and the chances to encounter stressful situations due to aggression are reduced.

The present results are obtained with animals of the BALB/c inbred strain. Before generalizing these recommendations, more strains must be tested in a similar way.

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